Alcaraz et al., http://www.jcb.org/cgi/content/full/jcb.201308031/DC1

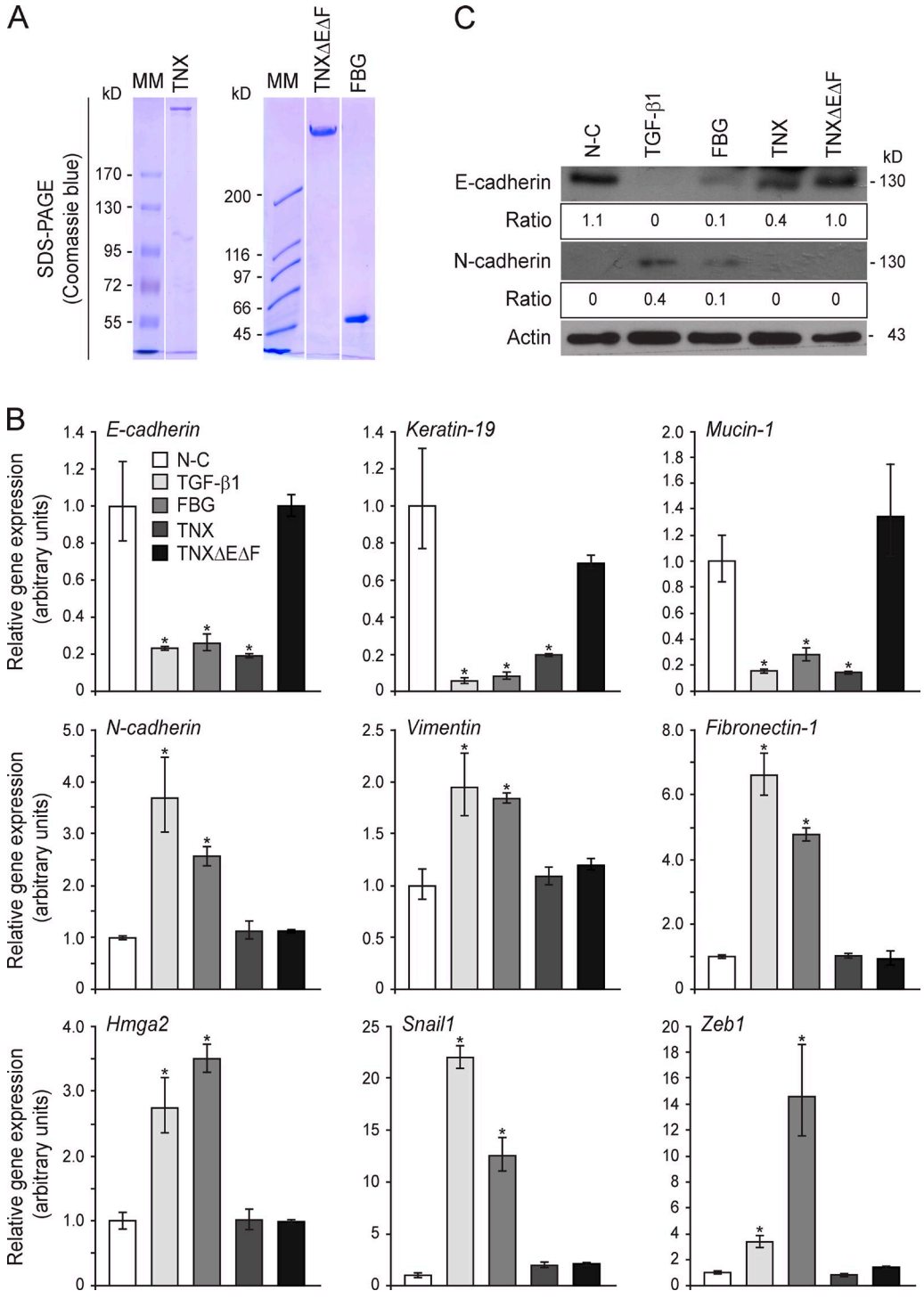

Figure S1. **Recombinant TNX fragments differentially regulate EMT in mammary epithelial cells.** (A) SDS-PAGE analysis of the purified recombinant proteins used in this study. Purified full-length TNX, the TNXΔEΔF fragment, and the FBG domain were loaded on 8% acrylamide gels under reducing conditions. MM, molecular mass markers. (B) Quantitative real-time RT-PCR analysis of known EMT markers and regulators in NMuMG cells cultured for 48 h onto noncoated dishes (N-C) or dishes coated with one of the following (111 pmol/cm^2) recombinant proteins: full-length TNX, a TNX derivative consisting of only the FNIII modules (TNXΔEΔF), or the FBG domain. As a control, NMuMG cells were stimulated for 48 h with 2 ng/ml of recombinant human TGF-β1. Error bars are means ± SD. *, $P < 0.05$ versus noncoated condition. (C) Immunoblots showing levels of E-cadherin and N-cadherin in NMuMG cells incubated for 48 h as described in B. Ratios of E-cadherin or N-cadherin to actin levels after densitometric analyses of the corresponding signals are indicated below each lane.

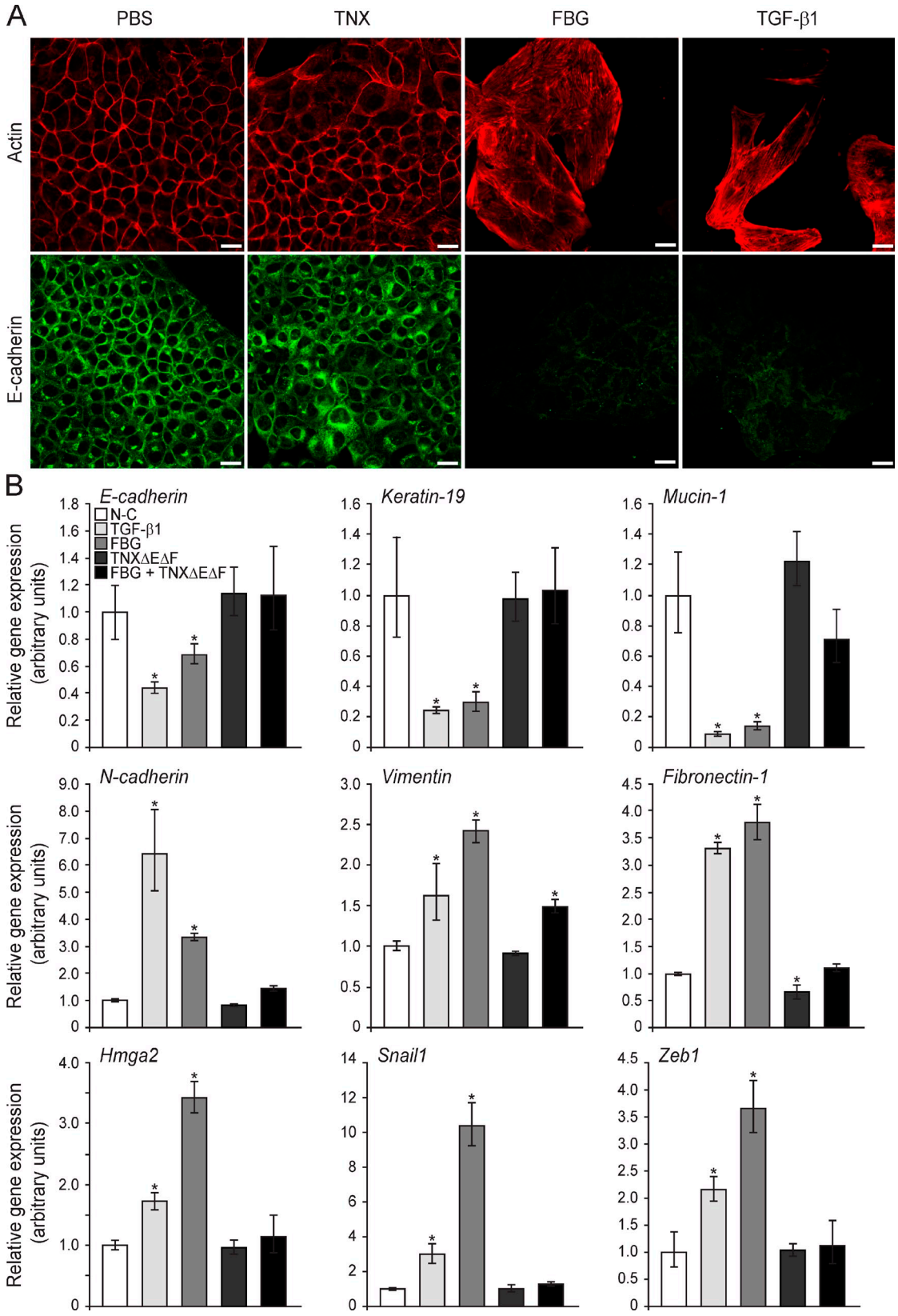

Figure S2. **The FNIII repeat–containing region of TNX molecule inhibits the EMT induced by FBG domain in mammary epithelial cells.** (A) F-actin direct fluorescence and E-cadherin indirect immunofluorescence performed in NMuMG cells cultured for 48 h in the presence of soluble recombinant TNX fragments (111 nM), the corresponding vehicle (PBS), or the recombinant human TGF-β1 (2 ng/ml). Bars, 15 µm. (B) Quantitative real-time RT-PCR analysis of known EMT markers and regulators in NMuMG cells cultured for 48 h in noncoated dishes (N-C) or dishes coated (111 pmol/cm^2) with the recombinant TNXΔEΔF fragment alone, the FBG domain alone, or both recombinant proteins (111 pmol/cm^2 each). As a control, NMuMG cells were stimulated for 48 h with 2 ng/ml of recombinant human TGF-β1. Error bars are means ± SD. *, $P < 0.05$ versus noncoated condition.

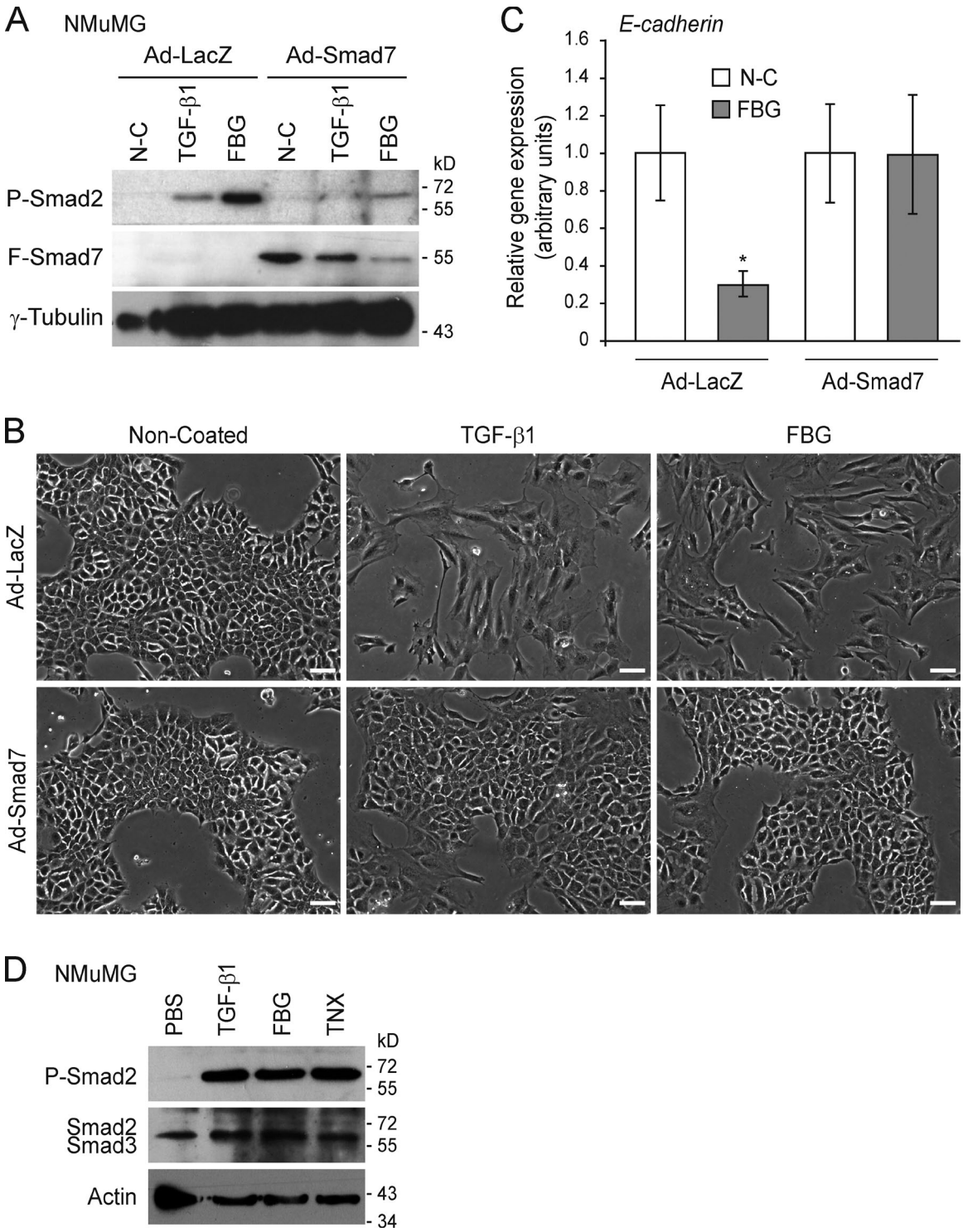

Figure S3. **The FBG domain of TNX employs the TβRI–Smad signaling pathway to trigger an EMT in mouse mammary epithelial cells.** (A) Immunoblot analyses showing levels of phospho-Smad2 (P-Smad2) and Flag-tagged Smad7 (F-Smad7) in NMuMG cells infected for 24 h with an adenovirus (multiplicity of infection 100) encoding either β-galactosidase (Ad-LacZ) or Smad7 (Ad-Smad7) and either seeded onto noncoated (N-C) dishes or dishes containing immobilized recombinant FBG domain (222 pmol/cm^2) and incubated for another 24 h or treated with 5 ng/ml of soluble TGF-β1 for the same time period. (B) Phase-contrast images of NMuMG cells infected for 24 h with LacZ- or Smad7-encoding adenovirus and either seeded onto noncoated or 222 pmol/cm^2 FBG-coated dishes and incubated for 36 h or treated with 5 ng/ml of soluble TGF-β1 for the same time period. Bars, 30 μm. (C) Quantitative real-time RT-PCR analysis of *E-cadherin* gene expression in NMuMG cells infected with LacZ- or Smad7-encoding adenovirus, seeded 24 h later onto noncoated or 222 pmol/cm^2 FBG-coated dishes, and cultured for another 24 h before isolation of total RNA. Error bars are means ± SD. *, $P < 0.05$ versus noncoated condition. (D) Immunoblot analyses showing levels of phospho-Smad2 (P-Smad2) and total-Smad2/3 proteins in NMuMG cells cultured for 3 h in the presence of soluble recombinant TNX fragments (111 nM), the corresponding vehicle (PBS), or the recombinant human TGF-β1 (2 ng/ml).

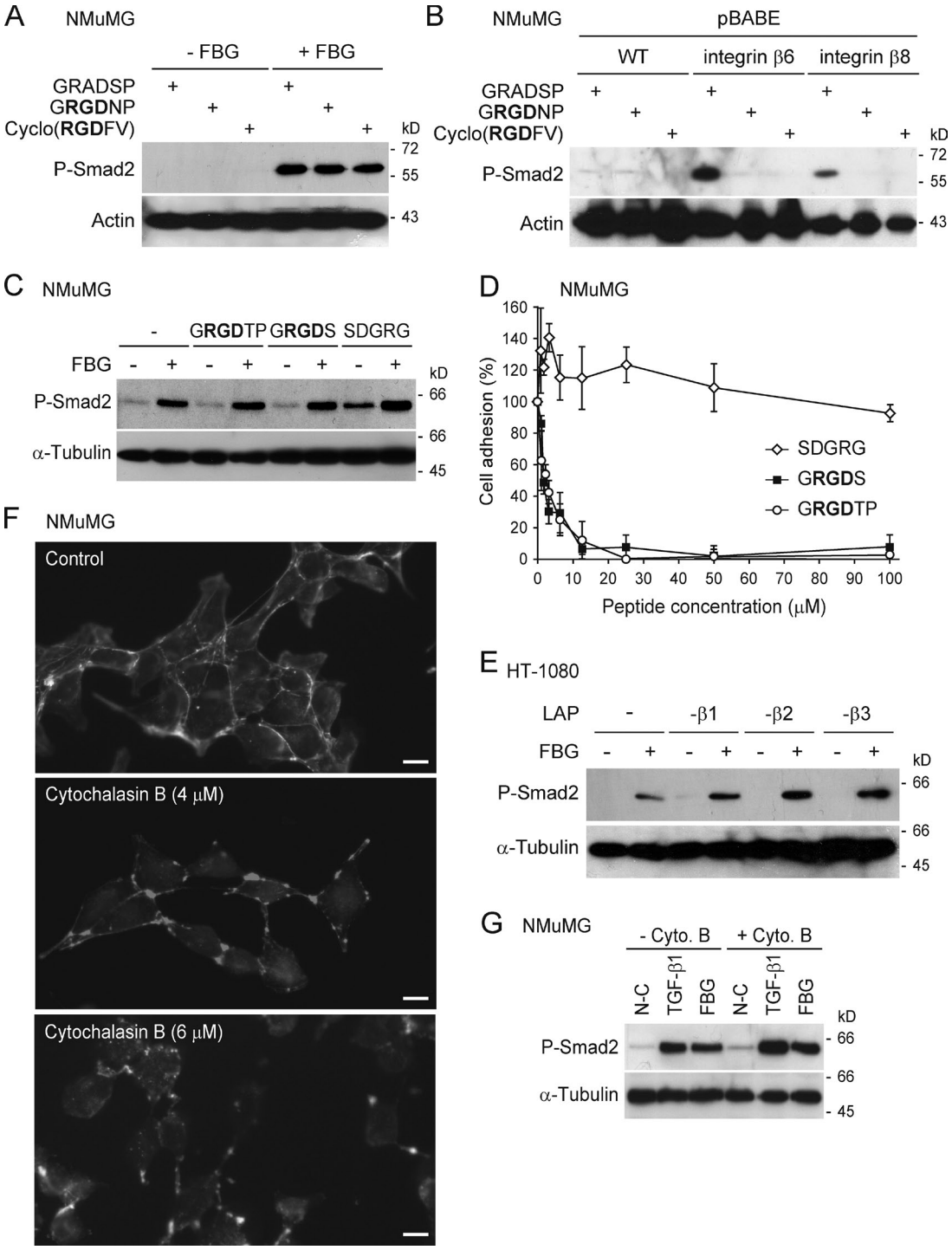

Figure S4. **FBG-mediated activation of latent TGF-β does not require either RGD-dependent integrins or an intact cytoskeleton.** (A) Level of phosphorylated Smad2 (P-Smad2) protein in NMuMG cells seeded (+) or not seeded (−) onto immobilized recombinant FBG domain (222 pmol/cm^2) and cultured for 3 h in the presence of control peptide (GRADSP) or linear (GRGDNP) or cyclic (Cyclo(RGDFV)) RGD peptide (100 μM). (B) Level of phospho-Smad2 in NMuMG cells stably infected with lentiviral particles containing either wild-type (WT) pBABE vector or a vector encoding the integrin β6 or integrin β8 chain and cultured for 3 h in the presence of control peptide (GRADSP) or linear (GRGDNP) or cyclic (Cyclo(RGDFV)) RGD peptide (100 μM). (C) Level of phosphorylated Smad2 in NMuMG cells seeded (+) or not seeded (−) onto immobilized recombinant FBG domain (222 pmol/cm^2) and cultured for 3 h in the absence (−) or presence of control (SDGRG) or linear (GRGDTP and GRGDS) RGD peptide (100 μM). (D) Adhesion assay of NMuMG cells seeded onto 10 μg/cm^2 of recombinant fibronectin type III domains 9 and 10 of TNX harboring an RGD sequence, in the presence of increasing concentrations of control (SDGRG) or linear RGD (GRGDTP and GRGDS) peptides. Results are expressed as the percentage of cell adhesion relative to the condition without peptide, and each point represents the mean of triplicate determinations with bars corresponding to standard error. (E) Level of phosphorylated Smad2 in HT-1080 cells transfected with the wild-type vector (−) or a vector expressing LAP(β1), LAP(β2), or LAP(β3) and cultured (+) or not cultured (−) with 25 μg/ml of soluble FBG domain for 3 h. (F) Direct F-actin fluorescence staining of NMuMG cells cultured for 30 min in the presence of cytochalasin B (at the indicated concentration) or vehicle (DMSO; control). Bars, 10 μm. (G) Level of phospho-Smad2 in NMuMG cells cultured for 3 h in a noncoated dish (N-C) or a dish containing immobilized recombinant FBG domain (222 pmol/cm^2) or stimulated with 5 ng/ml of soluble recombinant TGF-β1 in the presence of 1 μM cytochalasin B (Cyto. B) or vehicle (DMSO).

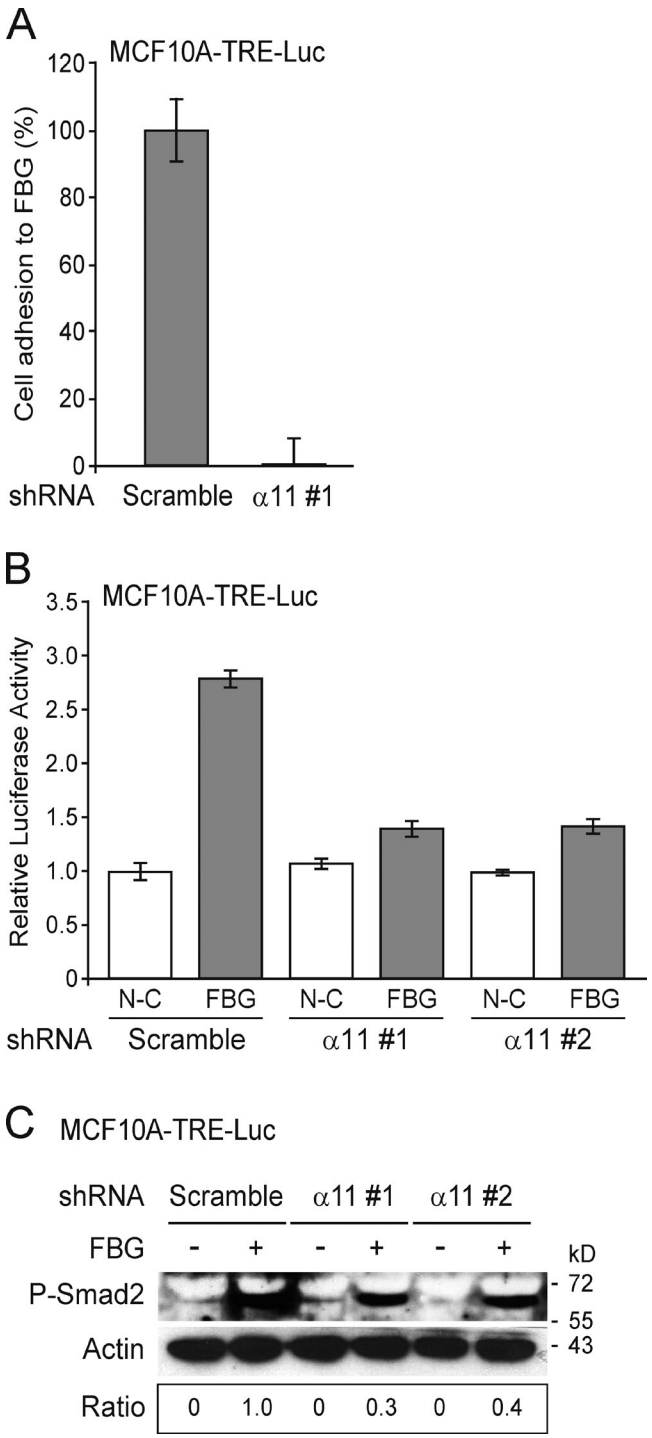

Figure S5. **Down-regulation of *ITGA11* expression impairs FBG-mediated activation of latent TGF-β in mammary epithelial cells.** (A) Adhesion analysis of MCF10A-TRE-Luc cells transiently expressing a scrambled shRNA or an shRNA targeting *ITGA11* mRNA (α11 #1) and cultured for 30 min after seeding onto wells coated with 222 pmol/cm^2 of recombinant FBG domain. Results represent percentage of cell adhesion relative to the scramble shRNA condition. (B) Firefly luciferase activity of MCF10A-TRE-Luc cells transiently transfected with a scrambled or *ITGA11*-targeting shRNA (α11 #1 or #2) and cultured for 16 h after seeding or not (N-C) onto coated recombinant FBG domain (222 pmol/cm^2). (C) Immunoblotting of phospho-Smad2 from MCF10A-TRE-Luc cells treated for 3 h as in B. Ratios of phospho-Smad2 (P-Smad2) to actin levels after densitometric analyses of the corresponding signals are indicated below. Error bars are means ± SD.